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Experimental Research on the Inhibitory Effect of IFN-Lambda 3 Combined with Sorafenib on the Growth of Liver Cancer Transplanted into Nude Mice

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Abstract: ***Objective:*** To investigate the effect of sorafenib combined with interferon-lambda 3 on the growth of liver cancer transplanted into nude mice. ***Methods:*** Female nude mice of 4-5 weeks of age that passed quarantine were selected and fed for 1-2 weeks before experimental operation. The cell suspension of human hepatoma cell line SMMC-7721 was inoculated into the right cervical axillary fossa with a syringe. The tumor-bearing mice were randomly divided into a control group and an experimental group. The control group received normal saline whereas the experimental group was further divided into three other groups: IFN-lambda 3 treatment group, sorafenib treatment group, and IFN-lambda 3 combined with sorafenib treatment group. The situation of nude mice was analyzed. At the end of the experiment, the volume of allogeneic transplanted tumor was measured, and the morphology of tumor cells, the expression of proliferating protein Ki-67, as well as the number of apoptotic cells were observed by hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC), and TUNEL staining. ***Results:*** The tumor cell volume of the IFN-lambda 3 treatment group, sorafenib treatment group, and IFN-lambda 3 combined with sorafenib treatment group decreased, which was statistically significant compared with the control group ($p < 0.05$). The increment rate of proliferating protein Ki-67 in the transplanted tumor tissue of the three drug groups was significantly lower than that of the control group ($p < 0.05$). IFN-lambda 3 combined with sorafenib had the greatest effect on the expression level of Ki-67 protein. Compared with the control group, the expression rate was significantly lower ($p < 0.05$). In terms of cell apoptosis, IFN-lambda 3 and/or sorafenib, as well as the combination of the two, showed statistically significant differences compared with the control group ($p < 0.05$). The rate of cell apoptosis was the highest in the IFN-lambda 3 combined with sorafenib group. ***Conclusion:*** IFN-lambda 3 combined with sorafenib can inhibit the growth and proliferation of human hepatocellular carcinoma cells in nude mice and promote the apoptosis of hepatocellular carcinoma cells, which proves that IFN-lambda 3 combined with sorafenib can treat hepatocellular carcinoma in vivo.

Keywords: Liver cancer; Sorafenib; IFN-lambda 3; Cell apoptosis

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1. Introduction

Liver cancer is one of the most common and highly malignant solid tumors of the gastrointestinal system around the world [1]. The clinical classification of tumor includes primary and secondary. According to the statistical data and pathological classification of European Association for Cancer Research (EACR), more than 90% of primary liver cancer is hepatocellular carcinoma (HCC) [2]. The characteristics of HCC which

include the difficulty in detecting its onset, long latent period, difficulty in early diagnosis, difficult to treat after diagnosing, rapid invasion and progress, postoperative recurrence and metastases, lack of effective intervention, as well as difficulty in technical transformation remain unchanged till now. Many bottleneck problems result in the high diagnosis rate and treatment rate of advanced HCC along with unsatisfactory treatment effect, which greatly reduces the quality of life of HCC patients. The 5-year recurrence rate after radical surgery is 50% to 70% [3], and the overall 5-year survival rate is about 5% to 30% [4]. Therefore, the diagnosis and treatment of HCC in patients are still common problems faced by clinicians around the world. Improving the curative effect and survival status of HCC patients, promoting the transformation from basic research to clinic trials, screening new intervention strategies, as well as making up for the weakness of current treatment methods have become the essential task of researchers and clinicians in the field of cancer [5,6]. The advent of sorafenib has opened up a new direction of molecular targeted therapy for liver cancer. At present, the recognized mechanism of action is that it plays an inhibitory role from the genesis of tumor to the new nutrient vessels required for the growth the tumor [7]. However, studies have found that the postoperative adjuvant therapy of sorafenib does not bring significant clinical benefits. At the same time, sorafenib adjuvant therapy shows obvious toxicity, side effects, and intolerance due to different types of liver injury [8]. Recent studies have found that the new interferon IFN-lambda 3 has strong antiviral, antitumor, and immunomodulatory effects [9]. However, in China, most patients with liver cancer also have hepatitis B. Therefore, it is not difficult to infer the hypothesis that the combination of molecular targeted drugs for HCC treatment and tumor immunotherapy can increase the treatment efficacy in HCC. This research further discusses the effect of IFN-lambda 3 combined with sorafenib on liver cancer transplanted nude mice in order to better provide experimental data support for further clinical application.

2. Materials and methods

2.1. Experimental materials

2.1.1. Animal

A total of 25 mice were selected from 4 to 5-week-old female nude mice that passed quarantine inspection to facilitate the establishment of tumor experimental model in nude mice. Before the experiment, the nude mice were in the adaptive feeding stage, and the laboratory was kept dry, sanitized, and quiet. The experimental operation was carried out after the mice were fed for 1-2 weeks.

2.1.2. Cells

The human hepatoma cell line, SMMC-7721, with positive expression of IFN lambda 3 receptor and strong sensitivity to sorafenib and/or IFN-lambda 3 was selected as the experimental hepatoma cell line. The cell line process was cultured in a sterile environment, and then the cells with excellent growth status were selected for transplantation into the nude mice.

2.1.3. Reagents and drugs

Sorafenib, IFN-lambda 3, hematoxylin and eosin (H&E) dye, anhydrous ethanol, xylene, hydrochloric acid, ammonia, and neutral gum; EDTA (pH 9.0 and pH 8.0) antigen repair solution, citric acid (pH 6.0) antigen repair solution, phosphate buffered saline (PBS), bovine serum albumin (BSA), normal rabbit serum, HRP-coupled goat anti-rat secondary antibody, HRP-coupled goat anti-rabbit secondary antibody, DAB chromogenic agent tissue kit, 10 × proteinase K storage solution, membrane breaking solution, TUNEL kit, and other related reagents and drugs.

2.2. Methods

2.2.1. Preparation for tumor cell transplantation

In the collection of human liver cancer cells, cells with logarithmic growth were selected. After the cells had been observed under the microscope, the configured PBS was selected to adjust the solution concentration of human liver cancer cells and maintain the solution concentration at 1.0×10^{6} / ml. The proportion of living cells of the cell line had to exceed 95%. The cell suspension was maintained at 0.1ml ~ 0.2ml for use.

2.2.2. Adaptive feeding of nude mice

The laboratory was kept dry, sanitized, and quiet. The indoor relative humidity was controlled at 30% to 70%, the indoor temperature was maintained at the most comfortable temperature condition for nude mice (20℃ to 26℃), and the indoor noise was controlled to below 60 dB. In addition, the sunshine time was modeled based on the actual sunshine situation, the number of indoor ventilation and air exchange was maintained at 10 to 15 times per hour, and the mice were fed for 1-2 weeks. A specially assigned person was responsible for the feeding process of the experimental mice. The weight of the mice was controlled at 10 g to 15 g each. All the food and use were via aseptic techniques.

2.2.3. Tumor cell transplantation

At the right cervical axillary region of a mouse, the subcutaneous area is loose, and the blood supply is abundant. This is convenient for cell inoculation. The operation was carried out in a sterile environment, and the prepared human liver cancer cell suspension was injected into the right lower neck axilla of the mice with a syringe. After inoculation, the experimental environment was strictly controlled to avoid infection at the inoculation site.

2.2.4. Observation

After inoculation, the changes at the inoculation site of the nude mice were observed daily. The mice were divided into four groups with 5 mice in each group by random number method when the diameter of the transplanted tumor nodule was about 3mm to 4mm. The control group, IFN-lambda 3 treatment group, sorafenib treatment group, and IFN-lambda 3 combined with sorafenib treatment group were established respectively. Treatment was given every day and the volume changes of the transplanted tumor cells were observed and recorded every 7 days. The tumor body was calculated as $V = a * b^2 / 0.525$ (a = length; b = width).

2.2.5. Tumor specimen observation

The experimental mice were killed using 10% chloral hydrate for 6 weeks. The transplanted tumor tissues in the right cervical axillary fossa of the experimental mice were stripped. Firstly, by naked eye, the color and texture of the tumor tissue, presence of a capsule, and its invasion into the surrounding tissues were observed. Paraffin sections were prepared for pathological H&E staining and immunohistochemical staining. In addition, some tissues were frozen in liquid nitrogen for TUNEL detection.

2.3. Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 22.0 software was used to analyze and process the relevant data. The counting data were expressed in percentage (%) and chi-square (χ^2) test was used for comparison. The measurement data were represented as $\bar{x} \pm s$, t-test was used for pairwise comparison,

and one-way ANOVA was used for multi-group comparison. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Changes in the tumor volume of the transplanted human hepatoma cell line

By regularly observing the tumor cell tissues every seven days, recording the volume changes of the transplanted tumor cells, and drawing a curve, it can be seen that the tumor cell volume decreased in the IFN-lambda 3 treatment group, sorafenib treatment group, and IFN-lambda 3 combined with sorafenib treatment group, which was statistically significant compared with the control group. Among them, IFN-lambda 3 combined with sorafenib treatment group had the largest reduction in the tumor cell volume with the most significant effect (**Figure 1**).

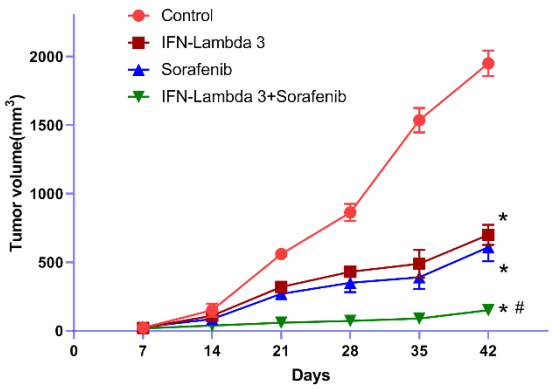

Figure 1. Change curve of the tumor volume of transplanted human hepatocellular carcinoma cell line

Note: (*) $p < 0.05$ versus control group; (#) $p < 0.05$ versus sorafenib or IFN-lambda 3 group

3.2. Changes of the transplanted tumor from the human hepatocarcinoma cell line in tumor-bearing nude mice through H&E staining microscopic examination

Under the microscope, the transplanted tumor tissue in the control group showed typical cancer tissue changes. In all three experimental groups, it can be appreciated that there was a significant reduction of cancer cells, with the IFN-lambda 3 combined with sorafenib treatment group being the most obvious and effective (**Figure 2**).

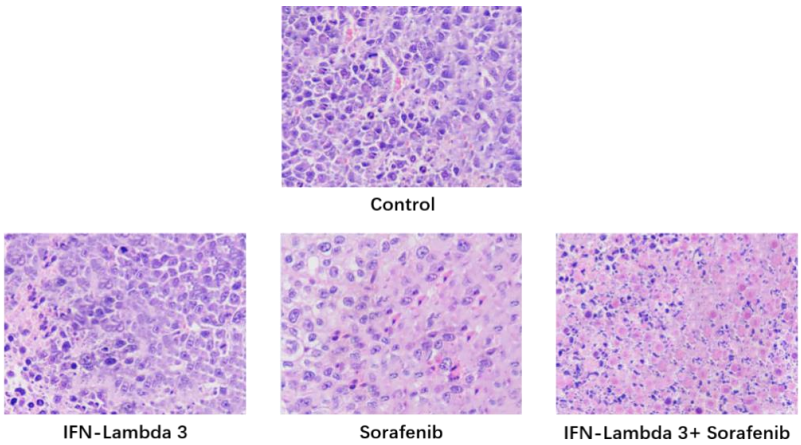

Figure 2. Changes of the transplanted tumor of human hepatocarcinoma cell line in tumor-bearing nude mice under H&E staining microscopic examination

3.3. Changes in the proliferative activity and apoptosis of human hepatocarcinoma cell line in tumor-bearing nude mice

IFN-lambda 3 and/or sorafenib, as well as a combination of the two reduced the positive expression rate of proliferating protein Ki-67 in the transplanted tumor, in which the difference was statistically significant compared with the control group ($p < 0.05$). Among them, the combination of sorafenib and IFN-lambda 3 had the greatest effect on the expression level of Ki-67 and significantly reduced the expression rate compared with the control group ($p < 0.05$) as shown in **Figure 3A**. TUNEL assay was used to detect the DNA fragments due to apoptosis in the transplanted tumor tissues. IFN-lambda 3 and/or sorafenib, as well as the combined intervention of the two had statistically significant differences compared with the control group ($p < 0.05$). Moreover, sorafenib combined with IFN-lambda 3 significantly induced apoptosis and increased the number of apoptotic cells (**Figure 3B**).

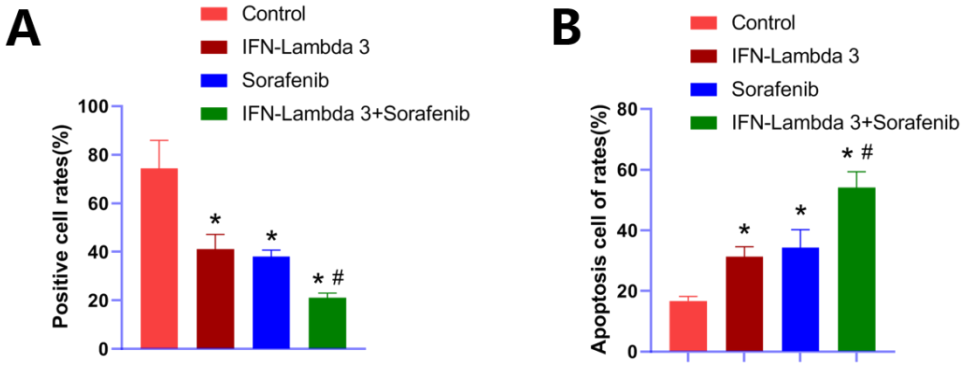

Figure 3. Changes in the proliferative activity and apoptosis of human hepatocarcinoma cell lines in tumor-bearing nude mice
Note: (*) $p < 0.05$ versus control group; (#) $p < 0.05$ versus sorafenib or IFN-lambda 3 Group

4. Discussion

Since the symptoms of primary liver cancer are not obvious during the early stage and the disease progresses rapidly, most patients are at the middle or late stage when they are diagnosed with the disease. In view of that, the effect of a simple surgical treatment is not ideal, thus radiotherapy and chemotherapy are usually the main treatment in clinical practice. Sorafenib is a multi-target therapeutic drug, which can directly inhibit the growth of tumor cells and block tumor angiogenesis. It is an effective drug for the treatment of cancer [10]. However, the clinical benefits from sorafenib do not meet the current severity and urgency of HCC treatment. Anyhow, the breakthrough of this new treatment concept has brought a new light to patients and clinicians. For this reason, sorafenib has been included in the fast-track channel for drug approval in many countries to treat advanced or partially end-stage HCC patients. Ever since its successful clinical application in the market, sorafenib is still the preferred treatment for patients with advanced HCC that cannot be surgically resected or that is complicated with distant organ invasion and metastases, despite doubts, challenges, and the impact of various new targeted drug development [11]. Therefore, looking for a supportive treatment regimen of sorafenib to improve the survival benefit of HCC patients has become a hot topic [12]. A large number of joint research experiments have begun, of which the most concerned is the interventional targeted therapy of transarterial chemoembolization (TACE) combined with sorafenib [13]. TACE blocks the main arterial supply of HCC, thus cutting off the supply of important nutrients and growth substances, to slow down the progress of HCC, promote tissue and cell ischemia as well as hypoxia in the tumor microenvironment, in addition to accelerate its death. Sorafenib seems to be able to effectively avoid the disadvantage of TACE. It directly reaches the tumor growth environment through the main artery to kill cancer cells, blocks the neovascular signal transduction pathway, and plays a complementary

sensitization effect of the combination of the two. However, it is a disappointment that the global multicenter, randomized controlled double-blind trials with high-level evidence-based medicine, phase II clinical SPACE studies and phase III clinical studies, have all declared negative results or failed in the middle, further confirming the complexity and intractable treatment of HCC. Researching and designing more feasible as well as effective treatment schemes are important, and they are the key for the treatment of HCC.

At present, interferon therapy has been approved by FDA to treat various diseases, including viral diseases, malignant tumors, and multiple sclerosis. Interferons are divided into three types according to their gene location and structural differences, namely type I, type II, and a newly discovered cytokine interferon, known as type III IFN family, including IFN-lambda 1, IFN-lambda 2, and IFN-lambda 3 (also known as interleukin IL-29, IL-28A, and IL-28B, respectively). Recent research has supplemented the member composition of type III IFN family, which is known as IFN-lambda 4. However, at present, there is more inclination to research about the functions related to IFN-Lambda 3 [14]. Previous studies have shown that IFN-alpha and sorafenib have antitumor effects on HCC in vitro and in vivo [15]. In this study, the xenotransplantation of human hepatoma cell line into nude mice was used to prove the feasibility of the combination of the two drugs. The results showed that IFN-lambda 3 combined with sorafenib could significantly inhibit the growth of transplanted tumor, reduce the positive expression of proliferating protein Ki-67, and promote the irreversible apoptosis of tumor cells. The results from this experimental research suggest that IFN-lambda 3 may effectively support the treatment of the current standard targeted therapeutic drug, sorafenib, for HCC patients. As a new cytokine drug, IFN-lambda 3 provides a new potential effective strategy for the treatment of HCC in combination with sorafenib. Its future use in clinical practice still requires high-quality multicenter randomized controlled research among HCC patients.

In conclusion, IFN-lambda 3 combined with sorafenib can inhibit the growth and proliferation of human hepatocellular carcinoma cells in nude mice, as well as promote the apoptosis of hepatocellular carcinoma cells, proving that IFN-lambda 3 combined with sorafenib can treat hepatocellular carcinoma in vivo.

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Disclosure statement

The authors declare that there is no conflict of interest.

References

[1] Hu J, Wang Q, Liu Y, 2021, Research Progress on Proteasome Subunits in Regulating Occurrence and Development of Hepatocellular Carcinoma. Zhejiang Da Xue Xue Bao Yi Xue Ban (Journal of Zhejiang University Medical Sciences), 50(3): 396-402. doi: 10.3724/zdxbyxb-2021-0146

[2] Kanwal F, Hoang T, Kramer JR, et al., 2011, Increasing Prevalence of HCC and Cirrhosis in Patients with Chronic Hepatitis C Virus Infection. Gastroenterology, 140(4): 1182-1188.e1. doi: 10.1053/j.gastro.2010.12.032

[3] Zhang W, Zhang B, Chen X, 2021, Adjuvant Treatment Strategy After Curative Resection for Hepatocellular Carcinoma. Frontiers of Medicine, 15(2): 155-169. doi: 10.1007/s11684-021-0848-3

[4] Wang J, Li J, Tang G, et al., 2021, Clinical Outcomes and Influencing Factors of PD-1/PD-L1 in Hepatocellular Carcinoma. Oncology Letters, 21(4): 279. doi: 10.3892/ol.2021.12540

[5] Waidmann O, 2018, Recent Developments with Immunotherapy for Hepatocellular Carcinoma. Expert Opinion on Biological Therapy, 18(8): 905-910. doi: 10.1080/14712598.2018.1499722

[6] DeLeon TT, Zhou Y, Nagalo BM, et al., 2018, Novel Immunotherapy Strategies for Hepatobiliary Cancers. Immunotherapy, 10(12): 1077-1091. doi: 10.2217/imt-2018-0024

[7] Zeng Z, Lu Q, Liu Y, et al., 2021, Effect of the Hypoxia Inducible Factor on sorafenib Resistance of Hepatocellular Carcinoma. Frontiers in Oncology, 11: 641522. doi: 10.3389/fonc.2021.641522

[8] Bruix J, Takayama T, Mazzaferro V, et al., 2015, Adjuvant Sorafenib for Hepatocellular Carcinoma After Resection or Ablation (STORM): A Phase 3, Randomised, Double-Blind, Placebo-Controlled Trial. Lancet Oncol, 16(13): 1344-1354. doi: 10.1016/S1470-2045(15)00198-9

[9] Qin S, Wang J, Zhou C, et al., 2019, The Influence of Interleukin 28B Polymorphisms on the Risk of Hepatocellular Carcinoma Among Patients with HBV or HCV Infection: An Updated Meta-Analysis. Medicine, 98(38): e17275. doi: 10.1097/MD.0000000000017275

[10] Muñoz-Martínez S, Iserte G, Sanduzzi-Zamparelli M, et al., 2021, Current Pharmacological Treatment of Hepatocellular Carcinoma. Current Opinion in Pharmacology, 60: 141-148. doi: 10.1016/j.coph.2021.07.009

[11] Qin S, Ren Z, Fen YH, et al., 2021, Atezolizumab Plus Bevacizumab Versus Sorafenib in the Chinese Subpopulation with Unresectable Hepatocellular Carcinoma: Phase 3 Randomized, Open-Label IMBrave150 Study. Liver Cancer, 10(4): 296-308. doi: 10.1159/000513486

[12] Cao Y, Ouyang T, Xiong F, et al., 2021, Efficacy of Apatinib in Patients with Sorafenib-Transarterial Chemoembolization Refractory Hepatocellular Carcinoma: A Retrospective Study. Hepatology International. doi: 10.1007/s12072-021-10198-3

[13] Hang L, Yan ZP, Hou ZH, et al., 2021, Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios as Predictors of Outcomes in Patients with Unresectable Hepatocellular Carcinoma Undergoing Transarterial Chemoembolization Plus Sorafenib. Front Mol Biosci, 8: 624366. doi: 10.3389/fmolb.2021.624366

[14] Subhajit R, Debarati GR, Anand B, et al., 2021, Functional Genetic Variants of the IFN-Λ3 (IL28B) Gene and Transcription Factor Interactions on Its Promoter. Cytokine, 142: 155491. doi: 10.1016/j.cyto.2021.155491

[15] Kusano H, Ogasawara S, Akiba J, et al., 2013, Antiproliferative Effects of Sorafenib and Pegylated IFNα2b on Human Liver Cancer Cells In Vitro And In Vivo. International Journal of Oncology, 42(6): 1897-1903. doi: 10.3892/ijo.2013.1904